

Figure 1

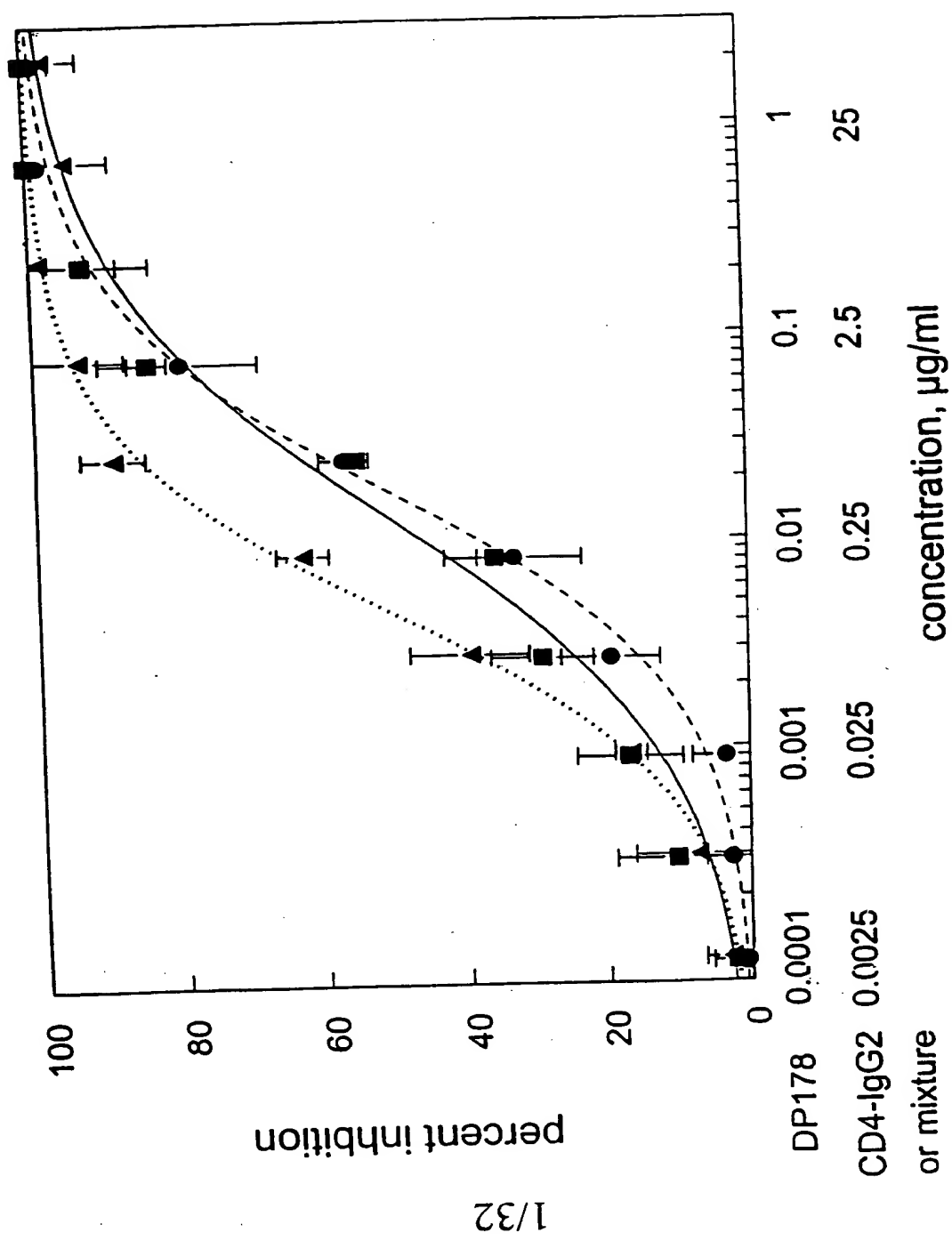


Figure 2

Percent Inhibition	Combination Index			
	CD4-IgG2:T-20 Mass Ratio			
	25:1 (low)	25:1 (high)	5:1	1:1
95	0.32	0.20	0.22	0.50
90	0.38	0.25	0.27	0.55
85	0.43	0.29	0.30	0.59
80	0.47	0.33	0.34	0.62
75	0.51	0.36	0.37	0.65
70	0.54	0.39	0.40	0.67
65	0.58	0.42	0.43	0.70
60	0.61	0.45	0.45	0.73
55	0.65	0.48	0.49	0.75
50	0.69	0.51	0.52	0.78

Figure 3

Percent Inhibition	T-20			CD4-IgG2		
	Concentration, $\mu\text{g/ml}$		Dose Reduction	Concentration, $\mu\text{g/ml}$		Dose Reduction
	Alone	Combination		Alone	Combination	
99	1.1	0.17	6.6	130	4.3	29
95	0.21	0.044	4.9	19	1.10	17
90	0.10	0.024	4.2	7.8	0.59	13
70	0.025	0.0076	3.3	1.6	0.19	8.4
50	0.011	0.0039	2.8	0.60	0.095	6.3

Figure 4A

Percent Inhibition	Combination Index	PRO 542				PA12				T-20			
		Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction	
		Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix
95	0.41	10	2.1	4.8	2.8	730	2.8	260	2.8	94	19	4.9	4.9
90	0.45	7.0	1.6	4.4	2.1	320	2.1	150	2.1	63	14	4.5	4.5
70	0.47	4.1	0.92	4.5	1.2	72	1.2	60	1.2	30	8.1	3.7	3.7
50	0.48	3.1	0.66	4.7	0.87	28	0.87	32	0.87	19	5.8	3.3	3.3

PRO 542, PA12 and T-20 were used in an approximate 1:1:10 molar concentration ratio.

Figure 4B

Percent Inhibition	Combination Index	PRO 542				PRO 140				T-20			
		Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction	
		Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix
95	0.40	8.5	1.9	4.5	1.9	19	1.0	19	19	140	17	8.2	
90	0.39	7.1	1.5	4.7	1.5	13	0.77	17	17	100	13	7.7	
70	0.37	5.3	0.87	6.1	0.87	7.2	0.46	16	16	57	7.7	7.4	
50	0.35	4.6	0.63	7.3	0.63	4.9	0.34	14	14	40	5.6	7.1	

PRO 542, PRO 140 and T-20 were used in an approximate 2:1:20 molar concentration ratio.

Figure 4C

Percent Inhibition	Combination Index	PRO 542				PRO 140				T-20			
		Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction	
		Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix
95	0.24	61	2.5	61	24	11.9	0.72	17	156	22	7.1		
90	0.22	32	1.4	32	23	8.4	0.40	21	96	13	7.4		
70	0.19	9.8	0.50	20	32	4.5	0.14	40	4.5	4.5	8.9		
50	0.18	4.7	0.26	18	41	3.0	0.074	23	2.3	2.3	10		

PRO 542, PRO 140 and T-20 were used in an approximate 4:1:30 molar concentration ratio.

Figure 4D

		PRO 140				T-20			
		Concentration, nM		Dose		Concentration, nM		Dose	
Percent Inhibition	Combination Index	Alone	Mix	Reduction	Alone	Alone	Mix	Reduction	Mix
95	0.56	12	1.8	6.7	156	55	2.8		
90	0.55	8.4	1.1	7.4	96	35	2.7		
70	0.55	4.5	0.51	8.8	40	16	2.5		
50	0.56	3.0	0.31	9.9	23	10	2.4		

PRO 140 and T-20 were used in an approximate 1:30 molar concentration ratio.

Triple Combination Synergistically Blocks HIV-1 Entry (I)

- T-20 alone ▲ PRO 542 alone ◆ PRO 140 alone ■ 3-way comb.
- T-20 in mix PRO 542 in mix - - - - PRO 140 in mix

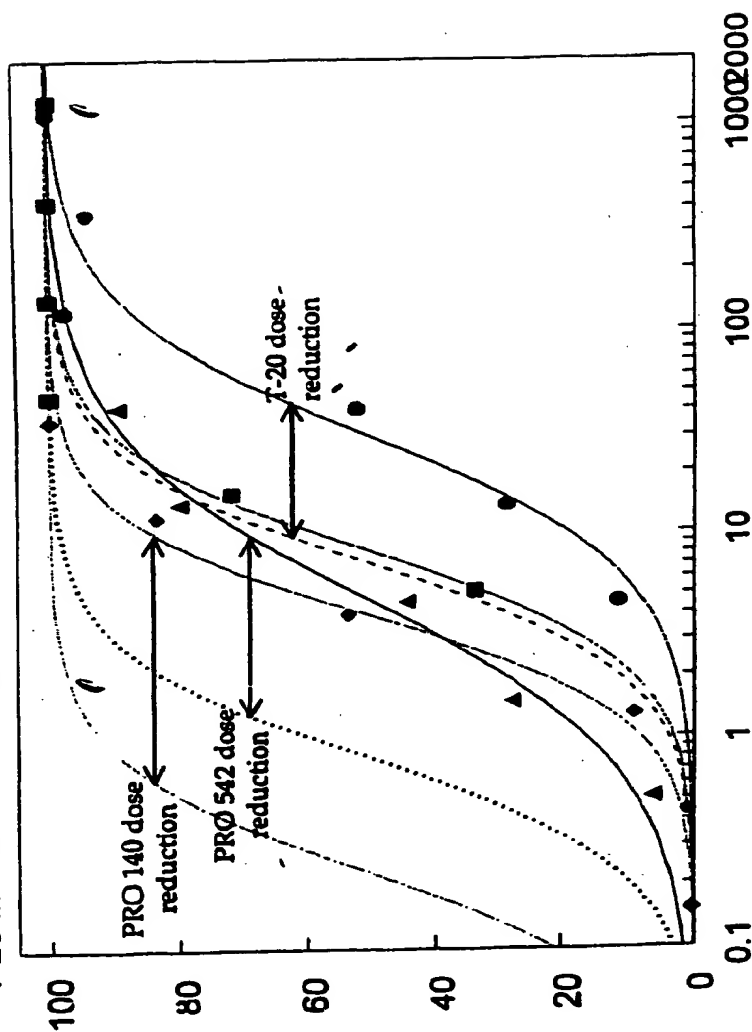


Figure 6

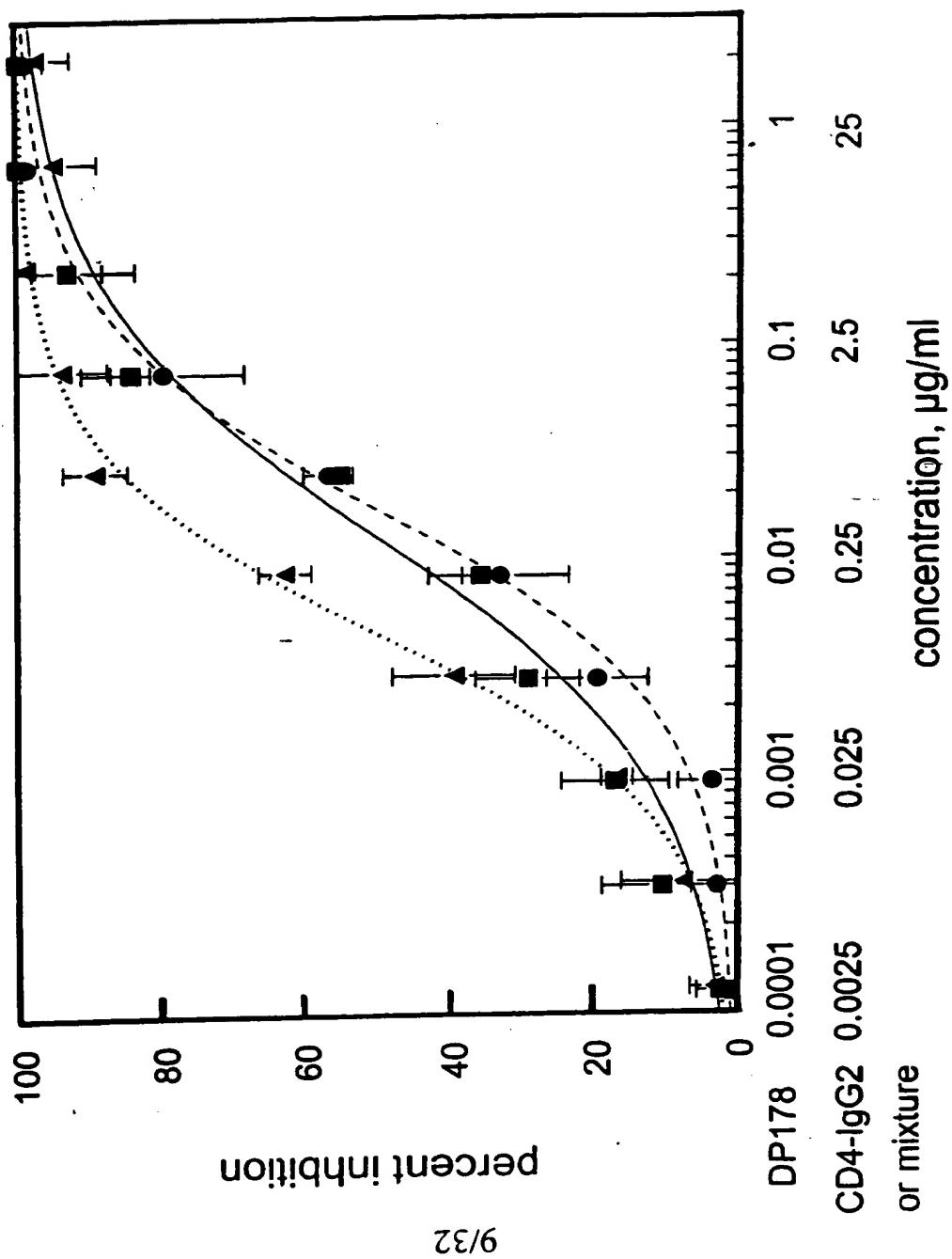


Figure 7

Combination Index				
CD4-IgG2:T-20 Mass Ratio				
Percent Inhibition	25:1 (low)	25:1 (high)	5:1	1:1
95	0.32	0.20	0.22	0.50
90	0.38	0.25	0.27	0.55
85	0.43	0.29	0.30	0.59
80	0.47	0.33	0.34	0.62
75	0.51	0.36	0.37	0.65
70	0.54	0.39	0.40	0.67
65	0.58	0.42	0.43	0.70
60	0.61	0.45	0.45	0.73
55	0.65	0.48	0.49	0.75
50	0.69	0.51	0.52	0.78

Figure 8

Percent Inhibition	T-20			CD4-IgG2		
	Concentration, $\mu\text{g/ml}$		Dose Reduction	Concentration, $\mu\text{g/ml}$		Dose Reduction
	Alone	Combination		Alone	Combination	
99	1.1	0.17	6.6	130	4.3	29
95	0.21	0.044	4.9	19	1.10	17
90	0.10	0.024	4.2	7.8	0.59	13
70	0.025	0.0076	3.3	1.6	0.19	8.4
50	0.011	0.0039	2.8	0.60	0.095	6.3

Figure 9

Assay (virus)	PRO 542:T-20 Molar Ratio	Percent Inhibition	Combination Index	PRO 542			T-20		
				Concentration, nM		Dose Reduction	Concentration, nM		Dose Reduction
				Alone	Mix		Alone	Mix	
Virus-cell fusion (JR-FL)	1:2	95	0.14	30	2.8	11	120	5.1	24
		90	0.18	12	1.5	8.0	45	2.6	17
		70	0.29	2.5	0.44	5.7	8.0	0.78	10
		50	0.39	0.92	0.21	4.4	2.7	0.37	7.3
Virus-cell fusion (DH'23)	1:2	95	0.36	65	11	5.9	123	20	6.2
		90	0.45	20	5.0	4.0	54	8.9	6.1
		70	0.76	2.4	1.2	2.0	12	2.1	5.7
		50	1.1	0.64	0.49	1.3	4.8	0.87	5.5
Cell-cell fusion (JR-FL)	1:2	95	0.36	35	6.3	5.6	73	11	6.6
		90	0.43	14	3.2	4.4	34	5.8	5.9
		70	0.61	2.9	0.94	3.1	8.5	1.7	5.0
		50	0.76	1.0	0.43	2.3	3.6	0.78	4.6
Cell-cell fusion (JR-FL)	1:10	95	0.27	28	1.4	20	58	12	4.8
		90	0.28	11	0.55	20	22	4.9	4.5
		70	0.31	2.3	0.11	21	3.8	0.97	3.9
		50	0.34	0.84	0.039	17	1.3	0.35	3.7
Cell-cell fusion (JR-FL)	1:50	95	0.33	47	0.84	56	120	37	3.2
		90	0.34	15	0.30	50	42	13	3.2
		70	0.36	1.8	0.045	40	6.1	2.0	3.0
		50	0.38	0.49	0.014	35	1.8	0.61	3.0

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Figure 10

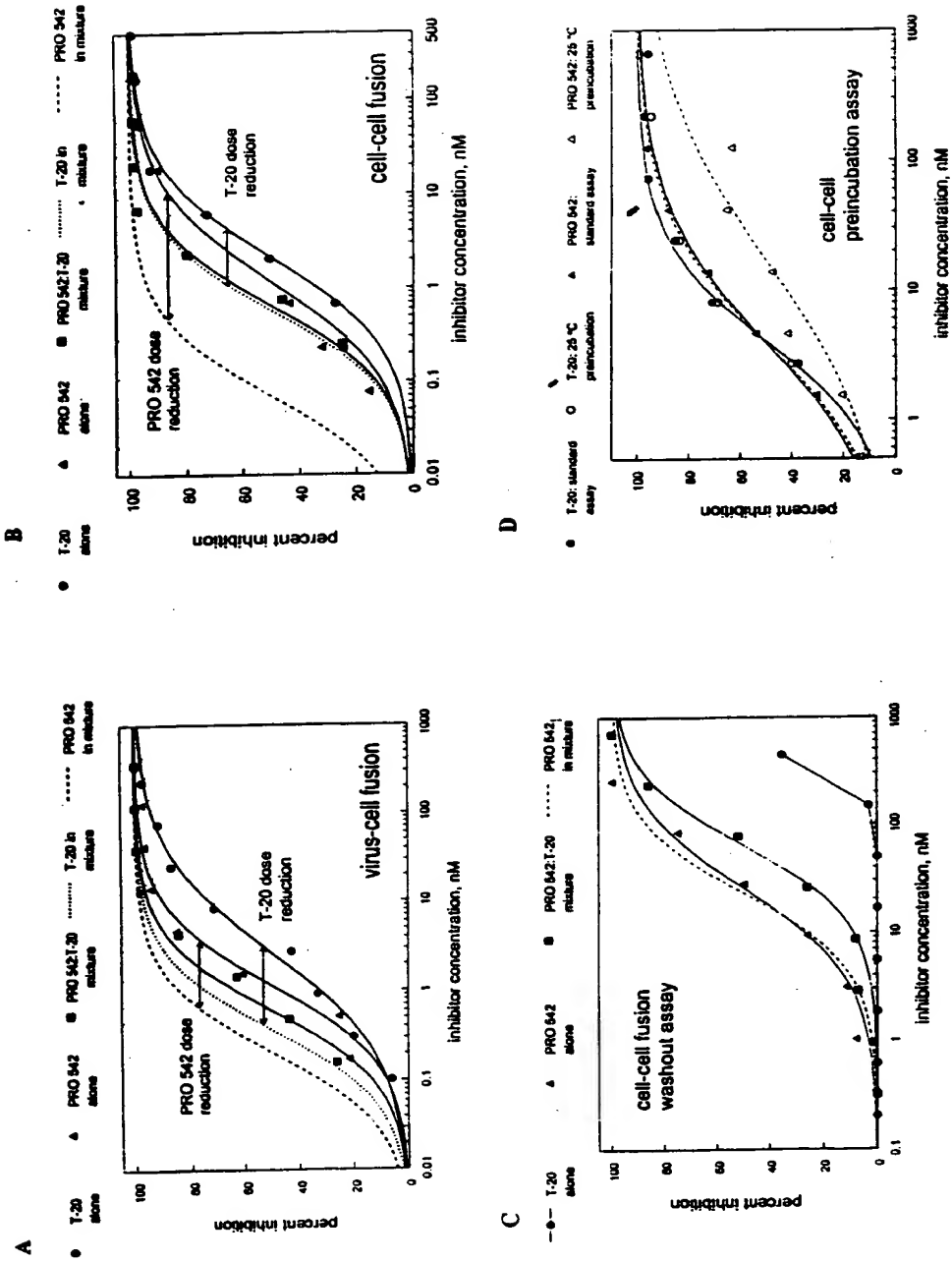


Figure 11

HIV-1 Entry Involves at Least Three Steps that Provide Promising Targets for Therapy

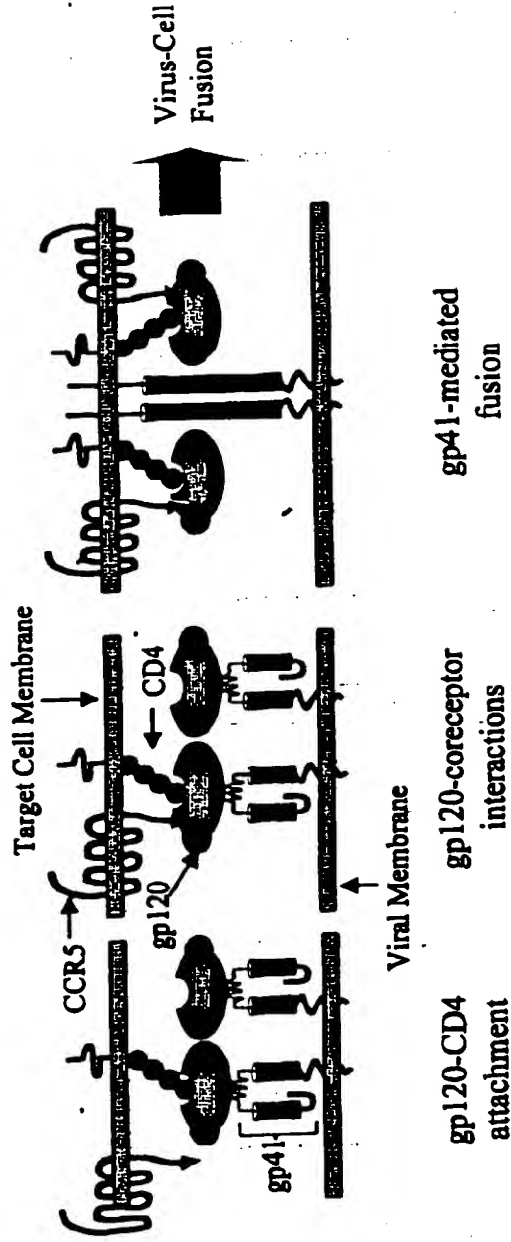


Figure 12

PRO 542 (CD4-IgG2)
attachment inhibitor

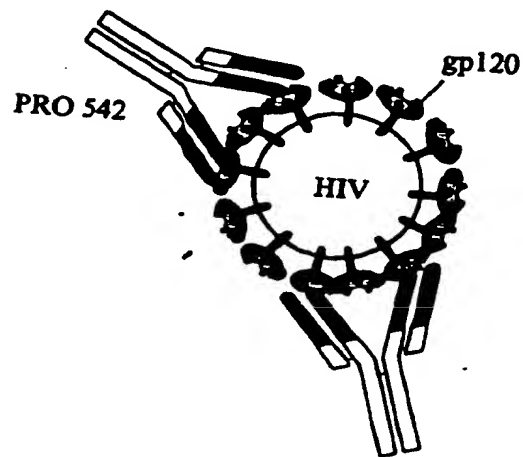
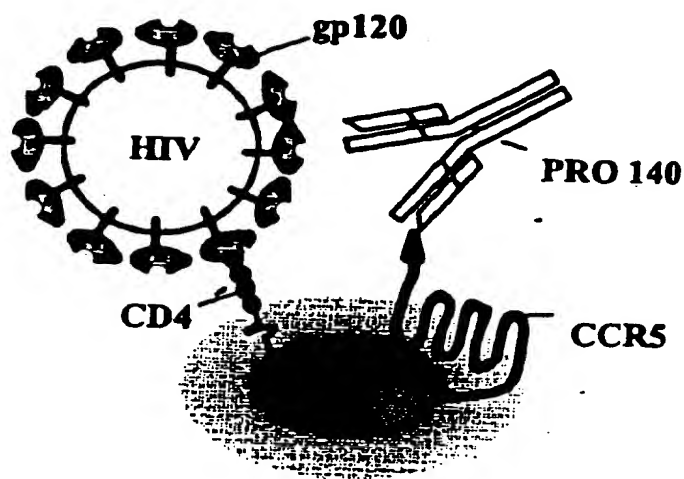


Figure 13

PRO 140
coreceptor inhibitor



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Figure 14

T-20
fusion inhibitor

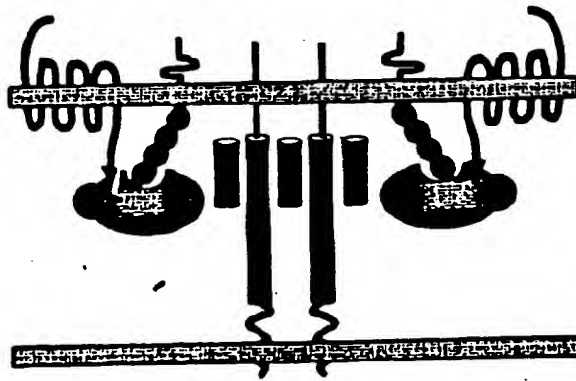


Figure 15

HIV-1 Virus-Cell Fusion Assay

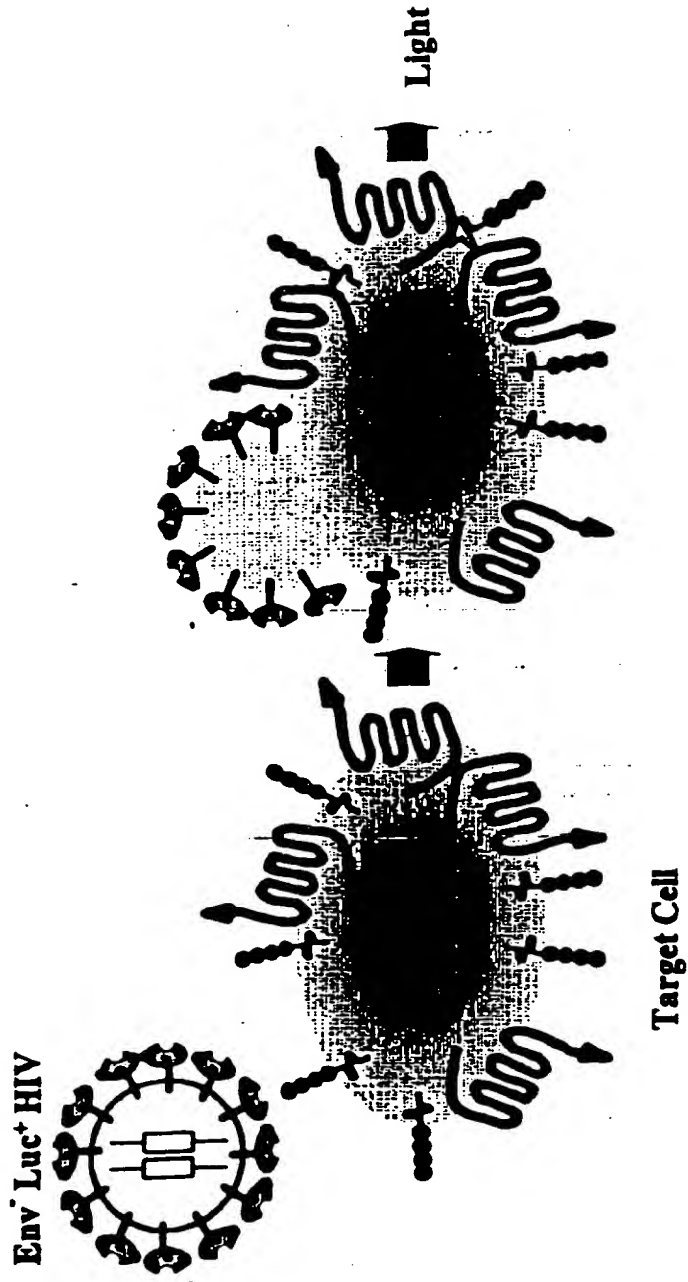


Figure 16

Synergistic Inhibition of Virus-Cell Fusion with PRO 542 and T-20 (I)

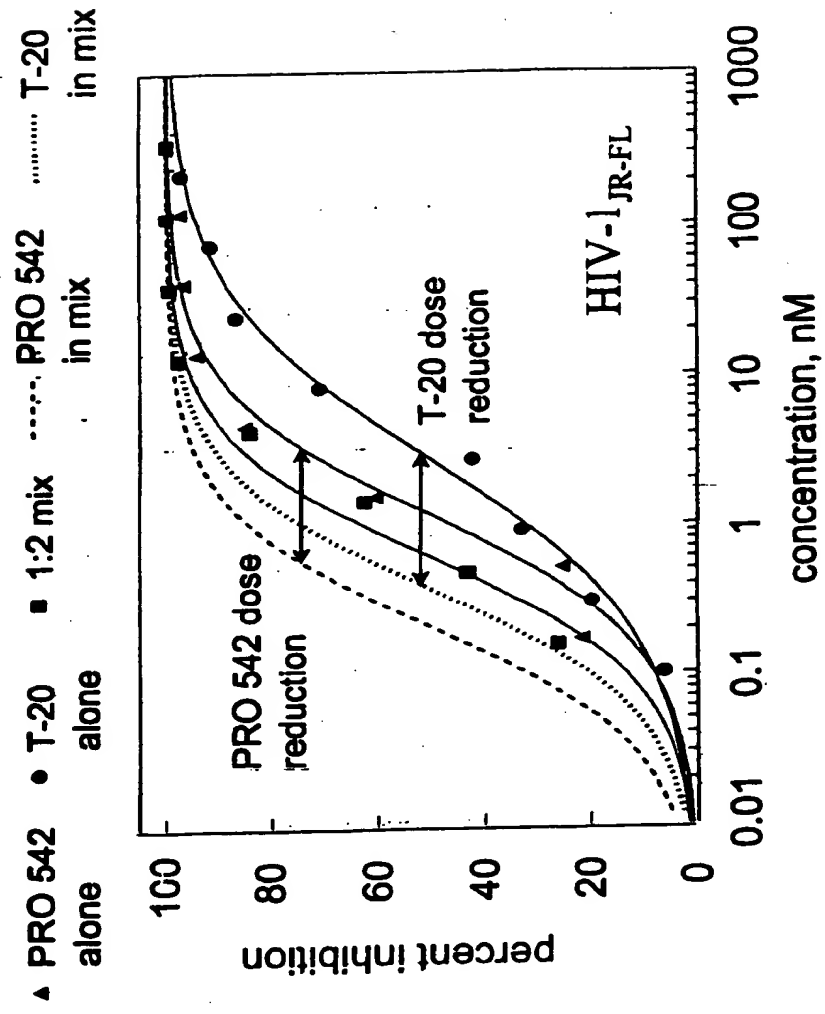


Figure 17

***Synergistic Inhibition of HIV-1 Virus-Cell-Fusion
with PRO 542 and T-20 (II)***

Percent Combination		Inhibitory Conc., nM		Dose Reduction	
Inhibition	Index	PRO 542	T-20	PRO 542	T-20
JR-FL 95	0.14	30	120	11	24
(R5) 90	0.18	12	45	8.0	17
70	0.29	2.5	8.0	5.7	10
50	0.39	0.92	2.7	4.4	7.3
DH123 95	0.36	65	123	5.9	6.2
(R5X4) 90	0.45	20	54	4.0	6.1
70	0.76	2.4	12	2.0	5.7
50	1.1	0.64	4.8	1.3	5.5

PRO 542 and T-20 were used in a 1:2 molar ratio

Figure 18

HIV-1 Cell-Cell Fusion Assay

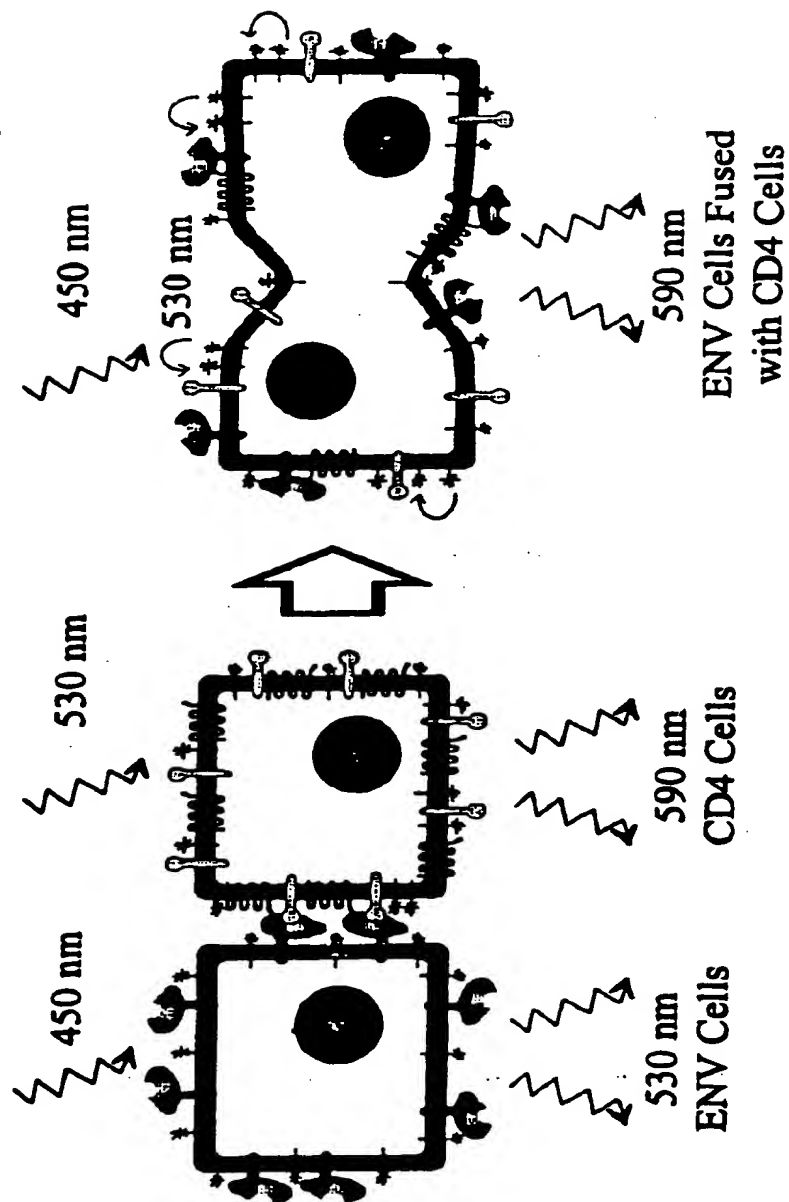


Figure 19

Synergistic Inhibition of Cell-Cell Fusion with PRO 542 and T-20 (I)

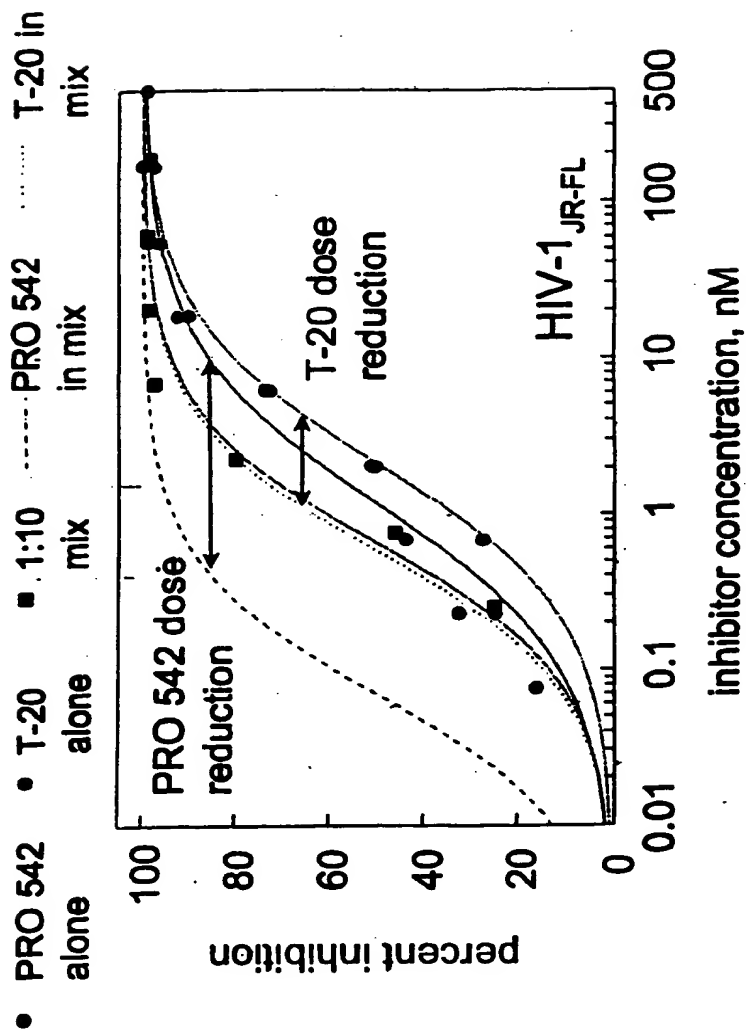


Figure 20

Synergistic Inhibition of HIV-1 Cell-Cell Fusion with PRO 542 and T-20 (II)

Conc. Ratio	Percent Inhibition	Combination Index	Inhibitory Conc, nM		Dose Reduction (fold)	
			PRO 542	T-20	PRO 542	T-20
1:2	95	0.32	95	47	17	4.9
	90	0.38	39	22	13	4.2
	50	0.69	3.0	2.5	6.2	2.8
1:10	95	0.27	28	58	20	4.8
	90	0.28	11	22	20	4.5
	50	0.34	0.84	1.3	22	3.7
1:50	95	0.33	47	120	56	3.2
	90	0.34	15	42	50	3.2
	50	0.38	0.49	1.8	35	3.0

Virus: HIV-1_{JR-FL}

Figure 21

PRO 140, PRO 542 and T-20 Triple Combination Synergistically Blocks HIV-1 Entry (I)

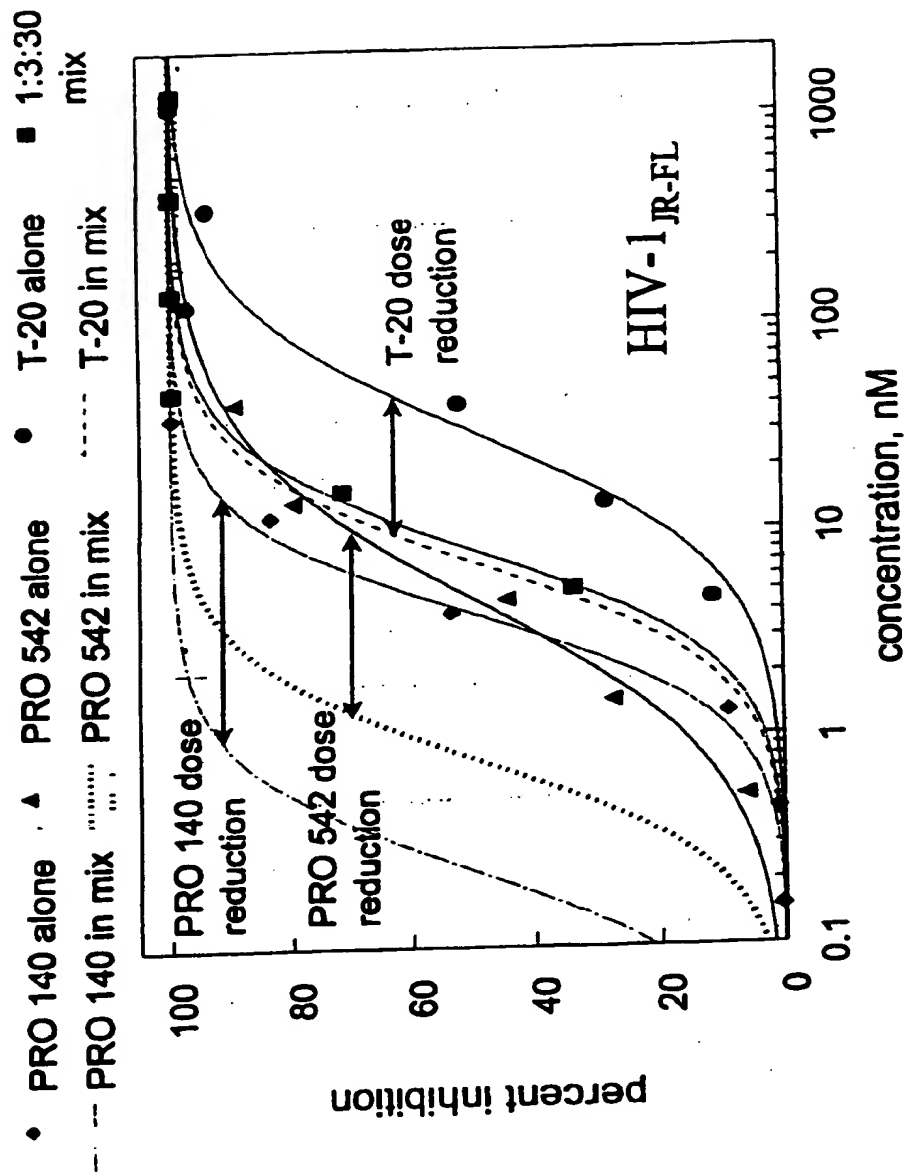


Figure 22

**PRO 140, PRO 542, T-20 Triple Combination
Synergistically Blocks HIV-1 Entry (II)**

Percent Inhibition	Combination Index	Inhibitory Conc, nM				Dose Reduction (fold)			
		PRO 140	PRO 542	T-20	PRO 140	PRO 542	T-20	PRO 140	T-20
95	0.24	24	61	160	17	12	7.1		
90	0.22	23	32	96	21	8.4	7.4		
70	0.19	20	9.8	40	32	4.5	8.9		
50	0.18	18	4.7	23	41	3.0	10		

Inhibition of HIV-1_{JR-FL} mediated cell-cell fusion with PRO 140, PRO 542 and T-20 used in a 1:3:30 molar ratio.

Figure 23

PRO 542 Does Not Potentiate T-20 Activity in the Absence of Coreceptor

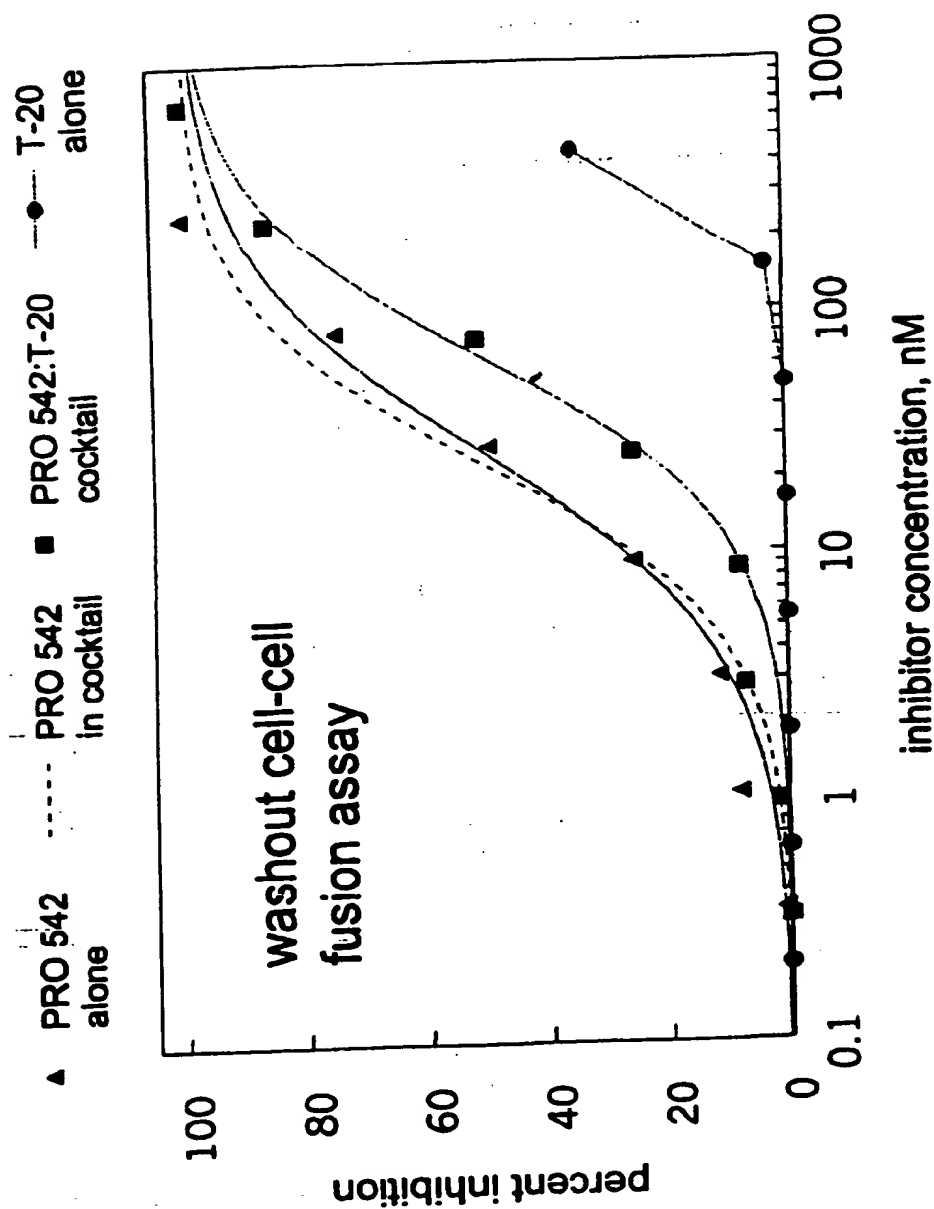


Figure 24

Formation of the Prehairpin Intermediate Requires CD4, Coreceptor and 37 °C (I)

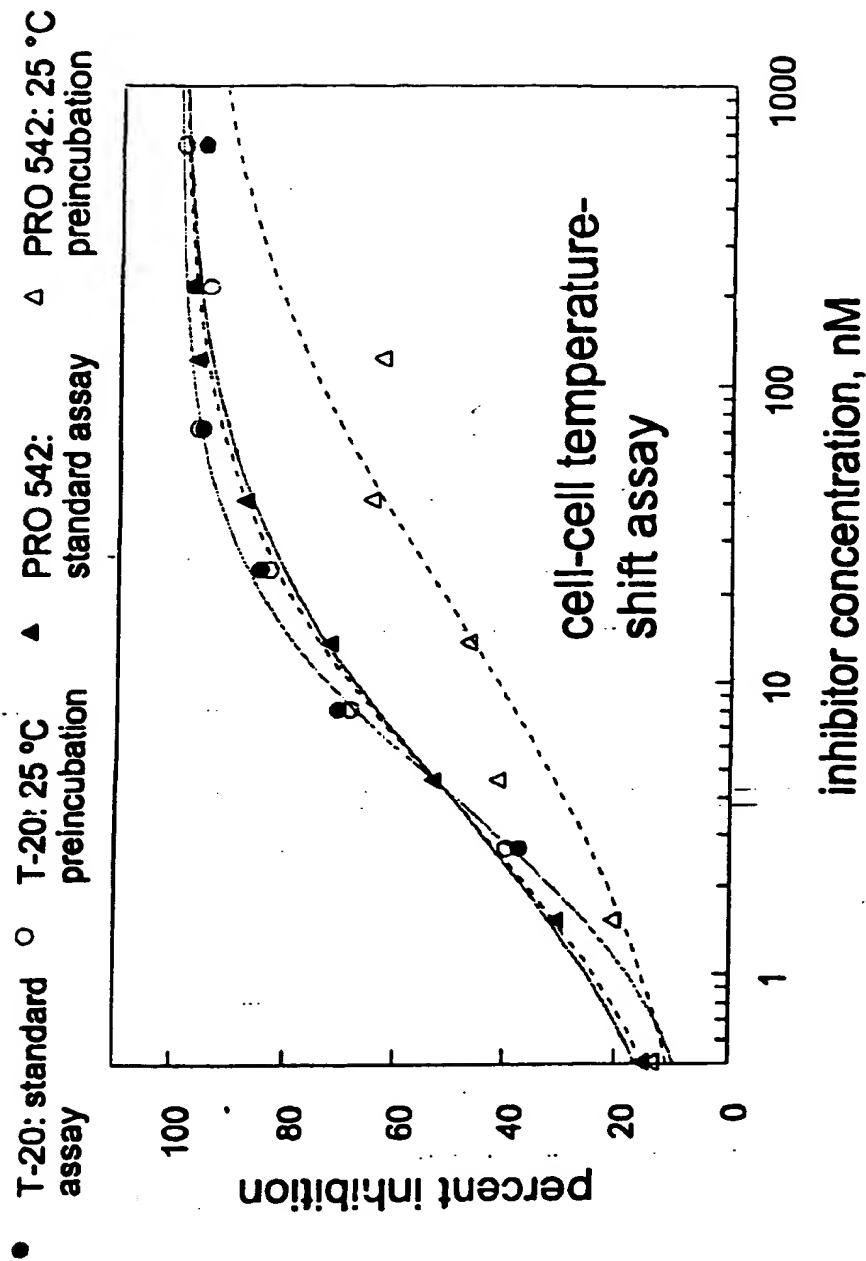


Figure 25

Formation of the Prehairpin Intermediate Requires CD4, Coreceptor and 37 °C (II)

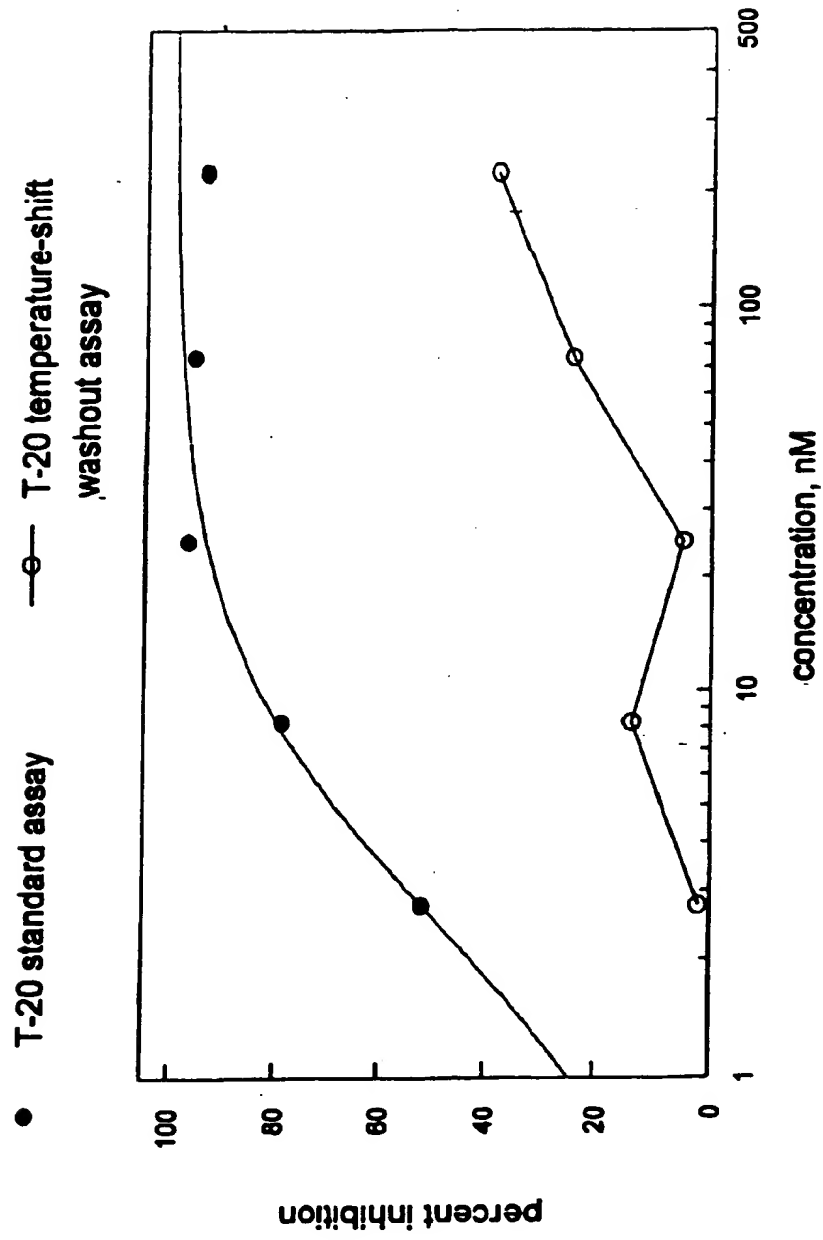


Figure 26

Possible Mechanism of Synergy: PRO 542 Increases the Half-Life of the T-20 Targets

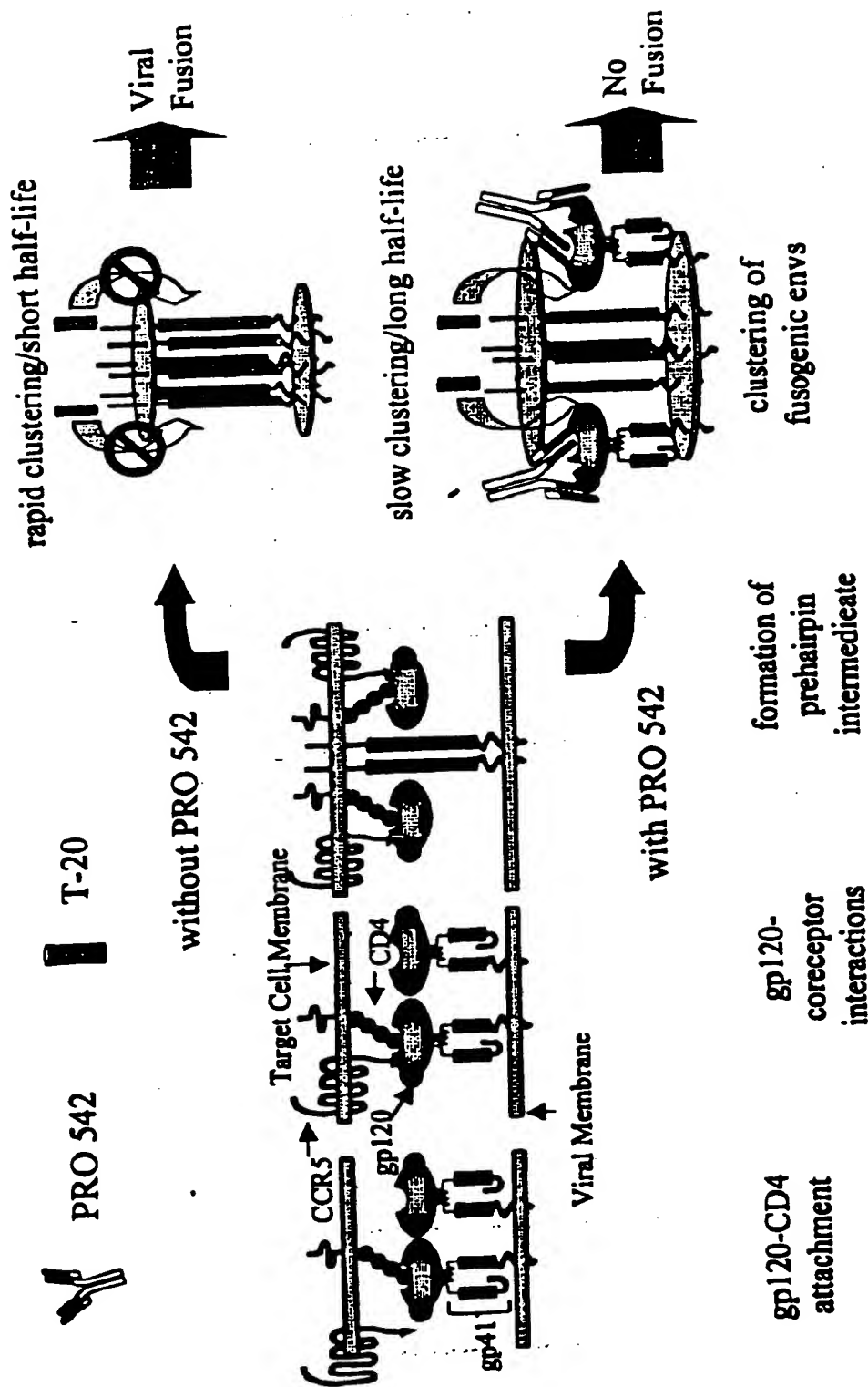


Figure 27

***Possible Mechanism of Synergy: PRO 542
Increases the Half-Life of the T-20 Targets***

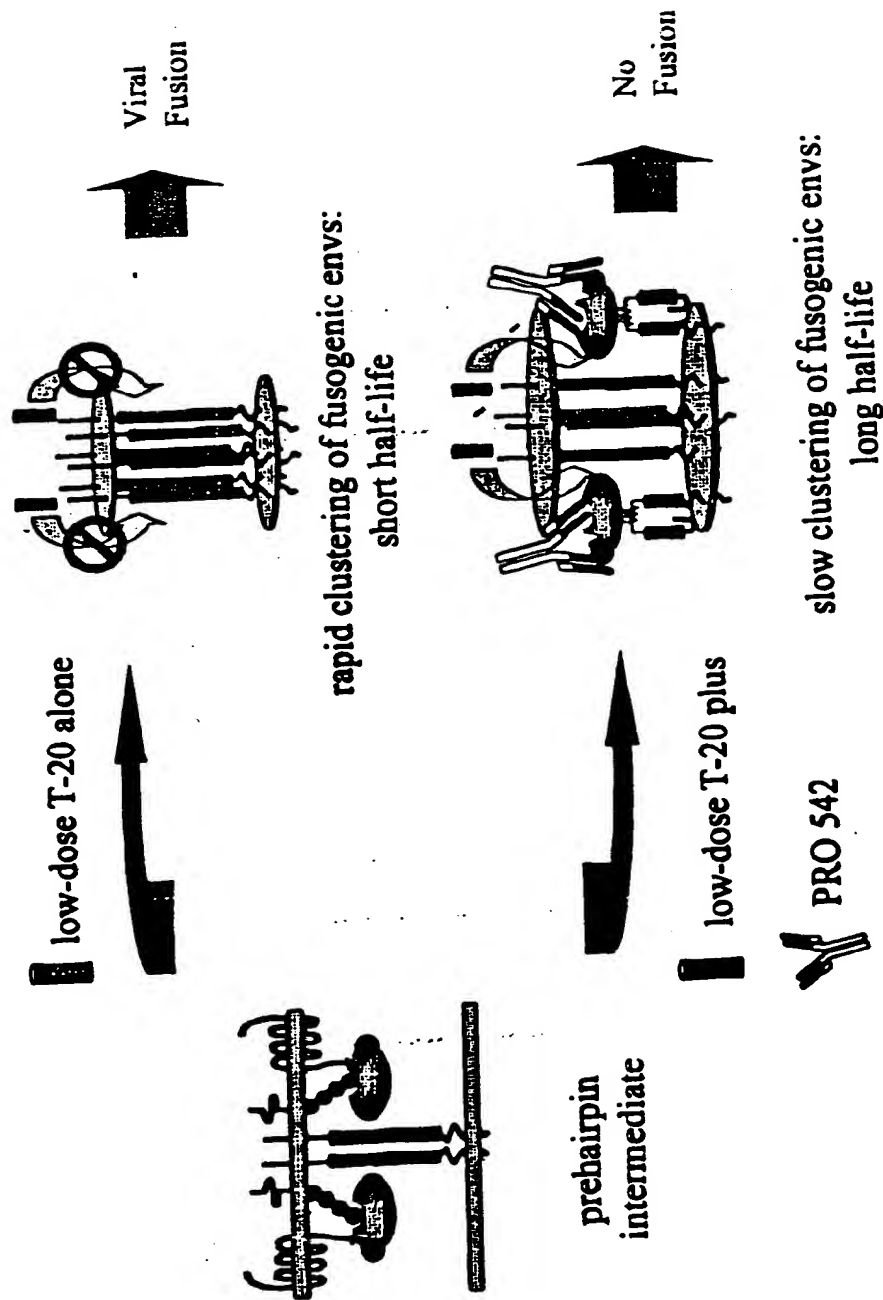


Figure 28

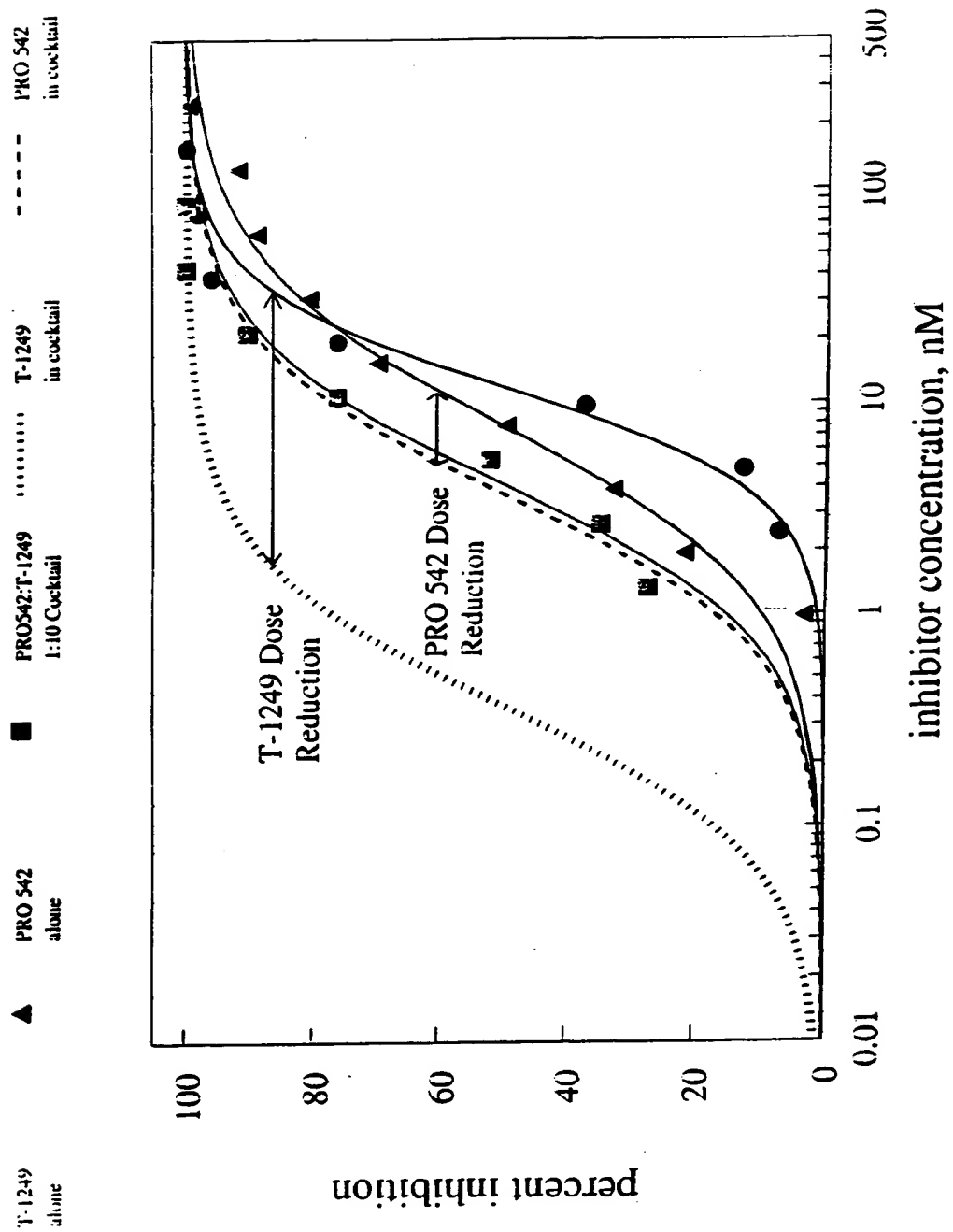


Figure 29

Fraction Inhibited	Dose PRO 542, nM (alone)		Dose PRO 542, nM (comb)		Dose T-1249, nM (alone)		Dose T-1249, nM (comb)		Combination Index		Dose Reduction	
	nM (alone)		nM (comb)		nM (alone)		nM (comb)		Index		PRO 542	
0.95	87.90		13.58		37.83		1.36		0.20		6.47	27.86
0.90	48.69		9.52		27.11		0.95		0.24		5.12	28.48
0.85	33.78		7.64		22.06		0.76		0.27		4.42	28.87
0.80	25.65		6.47		18.88		0.65		0.30		3.96	29.17
0.75	20.43		5.65		16.61		0.56		0.32		3.62	29.42
0.70	16.75		5.01		14.85		0.50		0.34		3.34	29.64
0.65	13.99		4.50		13.41		0.45		0.37		3.11	29.84
0.60	11.81		4.06		12.20		0.41		0.39		2.91	30.03
0.55	10.05		3.68		11.13		0.37		0.41		2.73	30.21
0.50	8.57		3.35		10.18		0.33		0.44		2.56	30.39